US Environmental Protection Agency
Office of Pesticide Programs

Office of Pesticide Programs
Microbiology Laboratory
Environmental Science Center, Ft. Meade, MD

Standard Operating Procedure for
Disinfectant Towelette Test Against Staphylococcus aureus, Pseudomonas aeruginosa, and Salmonella enterica

SOP Number: MB-09-05

Date Revised: 01-30-13

SOP Number	MB-09-05
Title	Disinfectant Towelette Test: Testing of *Staphylococcus aureus, Pseudomonas aeruginosa*, and *Salmonella enterica*
Scope	Describes the methodology used to determine the efficacy of towelette-based disinfectants against *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Salmonella enterica* on hard surfaces. The test is based on AOAC Method 961.02 (Germicidal Spray Products as Disinfectants). See 15.1.
Application	For product evaluations under the Antimicrobial Testing Program (ATP), a study protocol is developed which identifies the specific test conditions for a product sample such as contact time, neutralizers, etc. Although the default growth medium specified in this SOP is synthetic broth, other growth media (e.g., nutrient broth) may be specified by the study sponsor.

	Approval Date
SOP Developer:	_____ Print Name: _____
SOP Reviewer	_____ Print Name: _____
Quality Assurance Unit	_____ Print Name: _____
Branch Chief	_____ Print Name: _____

Date SOP issued:	
Controlled copy number:	
Date SOP withdrawn:	

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1. **Definitions**	Abbreviations/definitions are provided in the text.
2. **Health and Safety**	Follow procedures specified in SOP MB-01, Laboratory Biosafety. The Study Director and/or lead analyst should consult the Material Safety Data Sheet for specific hazards associated with products.
3. **Personnel Qualifications and Training**	Refer to SOP ADM-04, OPP Microbiology Laboratory Training.
4. **Instrument Calibration**	Refer to SOP EQ-01, EQ-02, EQ-03, EQ-04 and EQ-05 for details on method and frequency of calibration.
5. **Sample Handling and Storage**	Refer to SOP MB-22 and SOP COC-01.
6. **Quality Control**	For quality control purposes, the required information is documented on the appropriate form(s) (see section 14).
7. **Interferences**	1. Any disruption of the *Pseudomonas aeruginosa* pellicle resulting in the dropping or breaking of the pellicle in culture before or during its removal renders that culture unusable. 2. The carriers inside the Petri dishes should be dry prior to inoculation. Moisture can interfere with the concentration and drying of the inoculum on the glass slide carrier. 3. Any inoculated carrier that is wet at the conclusion of the carrier drying period should not be used.
8. **Non-conforming Data**	1. Sterility and/or viability controls do not yield expected results. 2. The mean log density for control carriers falls outside the specified range. Note: The prescribed minimum and maximum carrier counts also account for the addition of 5% organic soil to the inoculum. a. The mean *TestLD* for carriers inoculated with *S. aureus* and *P. aeruginosa* must be at least 5.0 (corresponding to a geometric mean density of 1.0×10^5) and not above 6.5 (corresponding to a geometric mean density of 3.2×10^6); a mean *TestLD* below 5.0 and above 6.5 invalidates the test, except for two retesting scenarios (outlined in the study protocol). b. The mean *TestLD* for carriers inoculated with *S. enterica* must be at least 4.0 (corresponding to a geometric mean density of 1.0×10^4) and not above 5.5 (corresponding to a geometric mean density of 3.2×10^5); a mean *TestLD* below 4.0 and above 5.5 invalidates the test, except for two retesting scenarios (outlined in the study protocol). 3. Management of non-conforming data will be specified in the study

	protocol; procedures will be consistent with SOP ADM-07, Non-Conformance Reports.
9. **Data Management**	Data will be archived consistent with SOP ADM-03, Records and Archives.
10. **Cautions**	There are time sensitive steps in this procedure including the use periods of the inoculated carriers and the test chemical.
11. **Special Apparatus and Materials**	1. *Subculture media*; use 20 mL aliquots (e.g., letheen broth, fluid thioglycollate medium, and Dey/Engley broth). Note: Commercial media made to conform to the recipes provided in AOAC Method 961.02 may be substituted. 2. *Test organisms*. *Pseudomonas aeruginosa* (ATCC No. 15442), *Staphylococcus aureus* (ATCC No. 6538) and *Salmonella enterica* (ATCC No. 10708) obtained directly from ATCC. 3. *Culture media*. Note: Commercial media (e.g., synthetic broth) made to conform to the recipes provided in AOAC Method 961.02 may be substituted. a. *Synthetic broth*. Use for (10 mL) daily transfers and (10 mL) final test cultures of *S. aureus, P. aeruginosa* and *S. enterica.* b. *Nutrient broth*. Alternatively, use for (10 mL) daily transfers and (10 mL) final test cultures of *P. aeruginosa.* 4. *Trypticase soy agar (TSA).* For use in propagation of the test organism to generate frozen cultures and as a plating medium for carrier enumeration. Alternately, TSA with 5% sheep blood (BAP) may be used. 5. *Sterile water*. Use reagent-grade water free of substances that interfere with analytical methods. Any method of preparation of reagent-grade water is acceptable provided that the requisite quality can be met. See Standard Methods for the Examination of Water and Wastewater and SOP QC-01, Quality Assurance of Purified Water for details on reagent-grade water. 6. *Carriers*. Glass Slide Carriers, 25 mm × 75 mm (or comparable size) borosilicate glass cover slips with number 4 thickness. Refer to SOP MB-03, Screening of Stainless Steel Cylinders, Porcelain Cylinders and Glass Slide Carriers Used in Disinfectant Efficacy Testing. 7. *Specialized glassware.* For primary and secondary subculture media, use autoclavable 38 × 100 mm glass tubes (Bellco Glass Inc., Vineland, NJ). Cap tubes with closures before sterilizing. 8. *Sterile surgical gloves*. For handling the towelette. 9. *Forceps*. For manipulating glass slides.

	10. *Micropipettes*. For performing culture transfers and serial dilutions. 11. *Positive displacement pipette.* With corresponding sterile tips able to deliver 0.01 mL. 12. *Timer*. For managing timed activities, any certified timer that can display time in seconds. 13. *Electronic Plate Scanning Device*. 3M™ Petrifilm™ Plate Reader, 3M Food Safety, St. Paul, MN, USA, Cat. No. 6499, or equivalent. 14. *3M™ Petrifilm™ Aerobic Count Plates*. 3M Food Safety, St. Paul, MN, USA, Cat. No. 6400.
12. **Procedure and Analysis**	One towelette is used to wipe ten carriers/slides. The area of the towelette used for wiping is folded and rotated so as to expose a new surface of the towelette for each carrier. The method may be altered to accommodate various towelette/carrier combinations (e.g., more than one towelette per set of ten slides). Prior to testing, perform the neutralization assay to determine if secondary subculture tubes are necessary. The Disinfectant Towelette Test Processing Sheet (see section 14) must be used for tracking testing activities.
12.1 Test Culture Preparation	a. Defrost a single cryovial (see Attachment 2) at room temperature and briefly vortex to mix. Add 10 µL of the thawed frozen stock (single use) to a tube containing 10 mL of growth medium (e.g., synthetic broth or nutrient broth), vortex, and incubate at 36 ± 1°C for 24 ± 2 h. One daily transfer is required prior to the inoculation of a final test culture. Daily cultures may be subcultured for up to 5 days; each daily culture may be used to generate a test culture. For *S. aureus* and *S. enterica* only, briefly vortex the 24 h cultures prior to transfer. b. For the final subculture transfer, inoculate a sufficient number of 20 × 150 mm tubes containing 10 mL growth medium (e.g., synthetic broth or nutrient broth) with 10 µL per tube of the 24 h culture then vortex to mix. Incubate 48-54 h at 36 ± 1°C. Do not disturb the 48-54 h test culture. Record all culture transfers on the Organism Culture Tracking Form (see section 14).
12.2 Carrier Inoculation	a. Inoculate approximately 80 carriers; 60 carriers are required for testing, 6 for control carrier counts, and 1 for the viability control. b. For *P. aeruginosa*, remove the pellicle from the broth either by decanting the liquid aseptically into a sterile tube, by gently aspirating the broth away from the pellicle using a pipette, or by vacuum removal. Avoid harvesting pellicle from the bottom of the tube. Transfer test culture after pellicle removal into sterile 25 ×

	150 mm test tubes (up to approximately 20 mL per tube) and visually inspect for pellicle fragments. Presence of pellicle in the final culture makes it unusable for testing. Proceed as below in 12.2c. c. For *S. aureus* and *S. enterica*, using a vortex-style mixer, mix 48-54 h test cultures 3-4 s and let stand 10 min at room temperature before continuing. Remove the upper portion of each culture (e.g., upper ¾ or approximately 7.5 mL), leaving behind any debris or clumps, and transfer to a sterile flask; pool cultures in the flask and swirl to mix. d. To achieve mean carrier counts within the appropriate range (see section 8), the final test culture may be diluted or concentrated. Dilution of the final test culture (e.g., one part culture plus one part sterile broth) should be made prior to the addition of the OSL to the inoculum. Concentration may be achieved using centrifugation (e.g. 5000g for 20 min.) and re-suspending the pellet in the appropriate volume of the sterile final test culture medium necessary to meet the carrier count range. Note: The use of a spectrophotometer to measure optical density (optical density at 650 nm) is recommended as a tool for assessing the need to dilute the final test culture. Always use sterile broth medium to calibrate the spectrophotometer. e. If organic burden is required for testing, the appropriate amount of organic burden is added to the pooled test culture prior to the inoculation of carriers. Swirl to mix. Aliquot a sufficient volume of culture into sterile test tubes. f. Use a calibrated positive displacement pipette to transfer 0.01 mL of the test culture onto the sterile test carrier in the Petri dish, at one end of the slide. Do not place inoculum in the middle of the slide. Vortex-mix the inoculum periodically during the inoculation of carriers. Immediately spread the inoculum uniformly over one third of the carrier surface using a sterile loop. Do not allow the inoculum to contact the edge of the glass slide carriers during the inoculation process. Cover dish immediately. g. Dry carriers in incubator at 36 ± 1°C for 30-40 min. Record the timed carrier inoculation activities on the Disinfectant Towelette Test Processing Sheet (see section 14). Perform efficacy testing within two hours of drying. h. After completion of all slide inoculations, thoroughly wipe the micropipette with 70% ethanol prior to removal from the BSC.
12.3 Enumeration of viable	a. Assay dried carriers in 2 sets of three carriers, one set immediately prior to conducting the efficacy test and one set immediately

	bacteria from carriers (control carrier counts)	following the test. Randomly select 6 inoculated carriers for carrier count analysis prior to efficacy testing. b. Place each of the inoculated, dried carriers in a 38 × 100 mm culture tube or sterile 50 mL polypropylene conical tube containing 20 mL of letheen broth. Vortex immediately – 60 ± 5 seconds for *P. aeruginosa* or 120 ± 5 seconds for *S. aureus* and *S. enterica*. Record the time of vortexing on the Disinfectant Towelette Test Processing Sheet (see section 14). c. After vortexing, briefly mix and make serial ten-fold dilutions in 9 mL dilution blanks of PBDW. Refer to the Disinfectant Towelette Test Carrier Counts Form (see section 14). If the serial dilutions are not made and plated immediately, keep the tubes at 2-5°C until this step can be done. Complete the dilutions and plating within 2 h after vortexing. Alternatively, pool the letheen broth from the tubes with the carriers after vortexing. Briefly vortex after pooling. Serially dilute and plate an aliquot of the pooled media (60 mL). The average carrier count per set will be calculated. d. Briefly vortex each serial dilution tube prior to plating. Plate 0.1 mL aliquots of appropriate dilutions in duplicate on TSA or BAP using spread plating. Plate appropriate dilutions to achieve colony counts in the range of 30-300 colony forming units (CFU) per plate. Spread inoculum evenly over the surface of the agar. Plates must be dry prior to incubation. e. Incubate plates (inverted) at 36 ± 1°C for up to 48 ± 2 h. f. Count colonies. Plates that have colony counts over 300 will be reported as TNTC. Record counts on the Disinfectant Towelette Test Carrier Counts Form (see section 14). See section 13 for data analysis. g. Alternatively, Petrifilm may be used for enumeration of bacterial organisms. Follow manufacturer's instructions for preparation and incubation of Petrifilm cards. *Note*: A culture purity check should be conducted on one dilution of one carrier.
12.4	Disinfectant Sample Preparation	a. Prepare disinfectant sample per SOP MB-22. b. Wipe the outside of the towelette packet or dispenser with 70% ethanol and allow to air dry prior to opening.
12.5	Test Procedure	a. Record timed events on the Disinfectant Towelette Test Time Recording Sheet for Carrier Transfers (see section 14). b. Wipe the outside of the towelette dispenser or packet with 70% ethanol and allow to air dry. c. Aseptically remove several towelettes before aseptically removing

	a towelette to initiate testing. Fold towelette in half lengthwise one to two times depending on the size. Beginning at the bottom, fold up towards the top five times. The following steps in the "procedure" section are more conveniently done with two analysts – one to manage the Petri dishes and slides, and the other to perform the wiping procedure. d. Remove the lid from the Petri dish and aseptically remove the inoculated slide and hold it firmly against the rim of the Petri dish. e. Wipe the slide back and forth three times lengthwise with the towelette for a total of six passes across the inoculum or as specified by the study sponsor. Wiping should be done within ±5 seconds of specified time. Place slide in Petri dish, close the lid, and allow slide to sit undisturbed for the contact time. Maintain the wiped carriers in a horizontal position. f. Repeat with four additional slides, folding the used section of the towelette in such a way as to expose a new surface for wiping each slide. g. After the fifth slide, unfold the vertical fold in the towelette and reverse the towelette so that the used surface of the towelette faces inward. Continue wiping an additional five slides, folding the towelette between each slide to expose a new surface. h. After the last slide of a set (typically 10 slides) has been wiped and the exposure time is complete, sequentially transfer each slide into the primary subculture tube containing the appropriate neutralizer within the ±5 second time limit. Drain the excess disinfectant from each slide, without touching the Petri dish, and transfer into the neutralizer tube. Perform transfers with sterile forceps. Place the inoculated/wiped end of the slide into the primary subculture medium. i. After the slide is deposited, recap the subculture tube and shake thoroughly. j. If a secondary subculture tube is deemed necessary to achieve neutralization, then transfer the carrier from the primary tube to a secondary tube. Within 25-60 min of the initial transfer, transfer the carriers using sterile forceps to a second subculture tube. Move the carriers in order but the movements do not have to be timed. Thoroughly shake the subculture tubes after all of the carriers have been transferred. k. Incubate all subculture tubes 48 ± 2 h at 36 ± 1°C.
12.6 Sterility and viability	a. Viability controls. Place 1 (or 2) dried inoculated untreated carrier(s) into separate tubes of the neutralizing subculture broth (if

controls	primary and secondary media are different). Incubate tubes with the efficacy test. b. Sterility controls. Place 1 (or 2) sterile untreated carrier(s) into separate tubes of the neutralizing subculture broth (if primary and secondary media are different). Incubate tube with the efficacy test.
12.7 Results	a. Gently shake each tube prior to recording results. Record results as + (growth) or 0 (no growth) as determined by presence or absence of turbidity, on the Disinfectant Towelette Test Results Sheet (see section 14). b. Viability control. Growth should occur in all tubes. c. Sterility control. Growth should not occur in any tubes. d. If secondary subculture tubes are used, the primary and secondary subculture tubes for each carrier represent a "carrier set." A positive result in either the primary or secondary subculture tube is considered a positive result for a carrier set. e. Specialized neutralizer/subculture medium such as Dey/Engley broth will not show turbidity; rather, the presence of pellicle at the surface of the medium (for *P. aeruginosa*) or a color change to the medium (yellow for growth of *S. aureus* or *S. enterica*) must be used to assess the results as a positive or negative outcome. i. Use viability controls for comparative determination of a positive tube. ii. If the product passes the performance standard, a minimum of 20% of the remaining negative tubes will be assayed for the presence of the test microbe using isolations streaks on TSA or BAP. Record preliminary results and conduct isolation streaks at 48 ± 2 h; however, continue to incubate negative tubes for up to an additional 24 hours to confirm the results.
12.8 Confirmatory Steps for Test Microbes	a. Confirm a minimum of three positive carrier sets per test. If there are less than three positive carriers, then confirm each carrier. If secondary subculture tubes are used and both tubes are positive in a carrier set, select only the tube with the carrier for confirmatory testing. b. For a test with greater than 20 positive carrier sets, confirm at least 20% by Gram staining, and a minimum of 4 positive carrier sets by Gram staining, solid media, and appropriate biochemical and antigenic analyses to ensure the identity of the organism. c. See Attachment 1 for Gram stain reactions, cell morphology, and colony characteristics on solid media.

	d. For additional confirmation steps refer to the appropriate Confirmation Flow Chart for *S. aureus*, *P. aeruginosa*, and *S. enterica* (see Attachment 3). e. If confirmatory testing determines that the identity of the unknown was not the test organism, annotate the positive entry (+) on the results sheet to indicate a contaminant was present.
13. **Data Analysis/ Calculations**	Calculations will be computed using a Microsoft Excel spreadsheet (see section 14). Both electronic and hard copies of the spreadsheet will be retained. Counts from 0 through 300 and their associated dilutions will be included in the calculations.
14. **Forms and Data Sheets**	1. Attachment 1: Typical Growth Characteristics of strains of *P. aeruginosa, S. aureus,* and *S. enterica.* 2. Attachment 2: Culture Initiation Flow Chart for *S. aureus*, *P. aeruginosa*, and *S. enterica.* 3. Attachment 3: Confirmation Flow Charts for *S. aureus, P. aeruginosa* and *S. enterica.* 4. Test Sheets. Test sheets are stored separately from the SOP under the following file names: Physical Screening of Carriers Record — MB-03_F1.docx Organism Culture Tracking Form (Frozen Stock Cultures) — MB-06_F2.docx Test Microbe Confirmation Sheet (Quality Control) — MB-06_F3.docx Disinfectant Towelette Test Carrier Counts Form — MB-09-05_F1.docx Disinfectant Towelette Test Time Recording Sheet for Carrier Transfers — MB-09-05_F2.docx Disinfectant Towelette Test Information Sheet — MB-09-05_F3.docx Disinfectant Towelette Test Results Sheet (1°/2°) — MB-09-05_F4.docx Disinfectant Towelette Test Results Sheet (1°) — MB-09-05_F5.docx Test Microbe Confirmation Sheet — MB-09-05_F6.docx Carrier Count Spreadsheet MS Excel spreadsheet: Carrier Count Template_DTT_v3 — MB-09-05_F7.xlsx Disinfectant Towelette Test Carrier Counts Form (Pooled Carriers) — MB-09-05_F8.docx

	Disinfectant Towelette Test Processing Sheet MB-09-05_F9.docx
15. **References**	1. Official Methods of Analysis. Revised 2013, publication pending. AOAC INTERNATIONAL, Gaithersburg, MD, (Method 961.02). 2. Krieg, Noel R. and Holt, John G. 1984. Bergey's Manual of Systematic Bacteriology Volume 1. Williams & Wilkins, Baltimore, MD. *P. aeruginosa* p. 164, *S. enterica* p. 447. 3. Sneath, P., Mair, N., Sharpe, M.E., and Holt, J. eds. 1986. Bergey's Manual of Systematic Bacteriology Volume 2. Williams & Wilkins, Baltimore, MD. *S. aureus* p. 1015. 4. Package Insert – Gram Stain Kit and Reagents. Becton, Dickinson and Company. Part no. 882020191JAA. Revision 07/2011. 5. Package Insert – Catalase Reagent Droppers. Becton, Dickinson and Company. Part no. L001237. Revision 06/2010. 6. Package Insert – Staphaurex Plus*. Remel. Part no. R30950102. Revised 11/23/07. 7. Package Insert – Oxidase Reagent Droppers. Becton, Dickinson and Company. Part no. L001133. Revision 06/2010. 8. Package Insert – Wellcolex* Colour Salmonella. Remel. Part no. R30858301. Revised 10/17/07

Attachment 1

Typical Growth Characteristics of strains of *P. aeruginosa*, *S. aureus*, and *S. enterica* (see ref. 15.2 and 15.3).

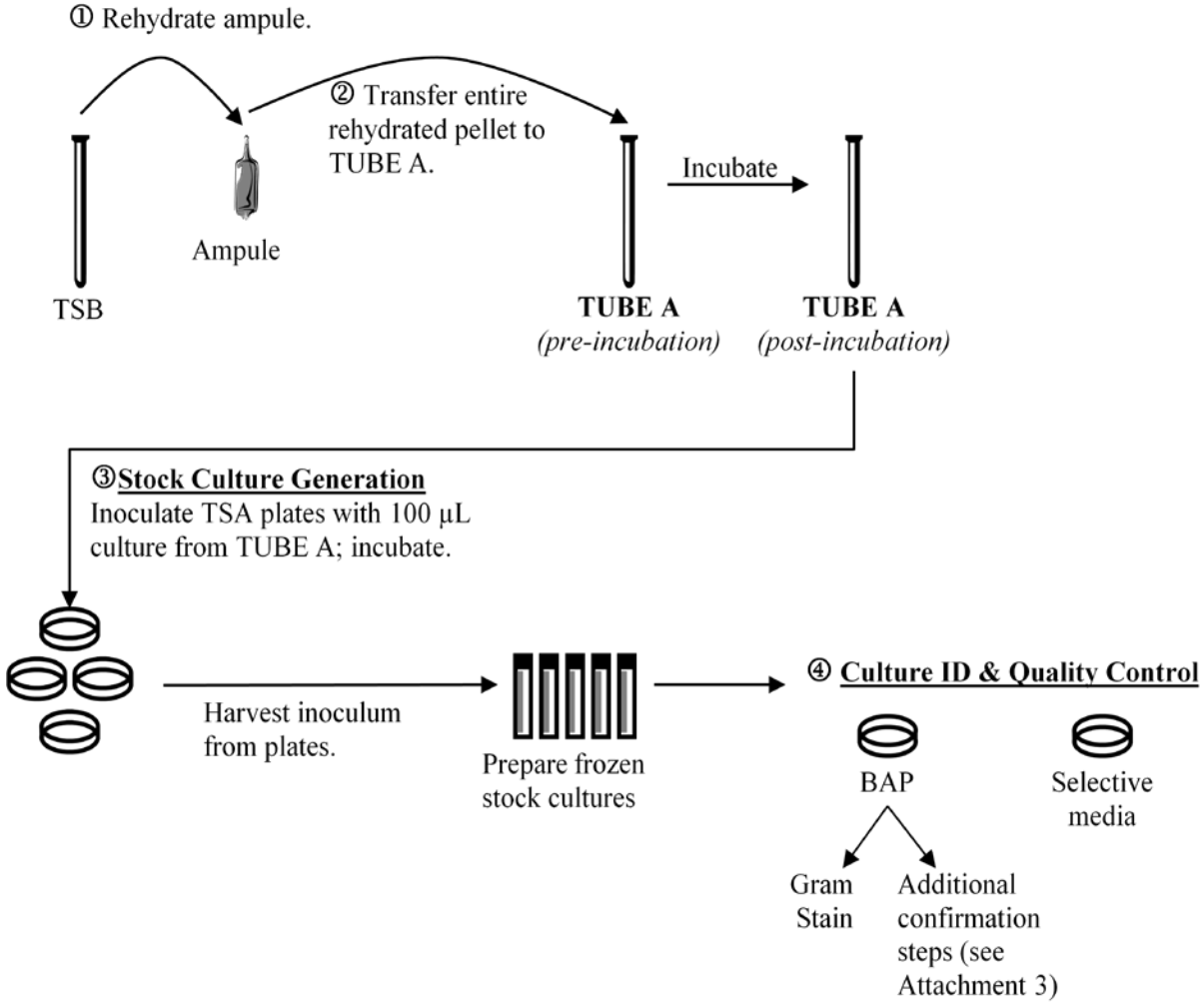
	*P. aeruginosa**	*S. aureus**	*S. enterica**
Gram stain reaction	(-)	(+)	(-)
Typical Growth Characteristics on Solid Media			
Mannitol Salt	No Growth	circular, small, yellow colonies, agar turning fluorescent yellow	N/A
Cetrimide	circular, small, initially opaque, turning fluorescent green over time; agar fluorescent yellowish green	No Growth	N/A
Xylose lysine deoxycholate (XLD) agar	N/A	N/A	Round, clear red colonies with black centers
Blood agar (BAP)	flat, opaque to off-white, round spreading (1), metallic sheen, slightly beta hemolytic	small, circular, yellow or white, glistening, beta hemolytic	entire, glistening, circular, smooth, translucent, low convex, non-hemolytic
Typical Microscopic Characteristics			
Cell dimensions	0.5-1.0 µm in diameter by 1.5-5.0 µm in length*	0.5-1.5 µm in diameter*	0.7-1.5 µm in diameter by 2.0-5.0 µm in length*
Cell appearance	straight or slightly curved rods, single polar flagella, rods formed in chains	spherical, occurring singly, in pairs and tetrads, sometimes forming irregular clusters	straight rods, peritrichous flagella

*After 24±2 hours

(1) Test organism may display three colony types: a) circular, undulate edge, convex, rough and opaque; b) circular, entire edge, convex, smooth and translucent; c) irregular, undulate edge, convex, rough, spreading, and translucent. Pyocyanin is not produced.

Attachment 2

Culture Initiation and Stock Culture Generation Flow Chart for *S. aureus*, *P. aeruginosa*, and *S. enterica*



A1. Preparation of Frozen Stock Cultures. Refer to SOP MB-02 for establishment of the organism control number.

a. Initiate new stock cultures from lyophilized cultures of *Pseudomonas aeruginosa* (ATCC 15442), *Staphylococcus aureus* (ATCC 6538), and *Salmonella enterica* (ATCC 10708) from ATCC within 18 months.

b. Open ampule of freeze dried organism as indicated by ATCC. Using a tube containing 5-6 mL of TSB, aseptically withdraw 0.5 to 1.0 mL and rehydrate the lyophilized culture. Aseptically transfer the entire rehydrated pellet back into the original tube of broth designated as "TUBE A". Mix well.

c. Incubate broth culture (TUBE A) at 36 ± 1ºC for 24 ± 2 hours. Record all

manipulations on the Organism Culture Tracking Form (see section 14).

d. Using a sterile spreader, inoculate a sufficient number of TSA plates (e.g., 5 to 10 plates per organism) with 100 µL each of the culture. Incubate plates at 36 ± 1°C for 24 ± 2 h.

e. Following incubation, add 5 mL cryoprotectant solution (TSB with 15% v/v glycerol) to the surface of each agar plate. Re-suspend the cells in this solution using a sterile spreader or a sterile swab and aspirate the cell suspension from the surface of the agar. Transfer the suspension into a sterile vessel. Repeat by adding another 5 mL of cryoprotectant to the agar plates, re-suspend the cells, aspirate the suspension and pool with the initial cell suspension.

f. Mix the pooled contents of the vessel thoroughly. Immediately after mixing, dispense approximately 1.0 mL aliquots into cryovials (e.g., 1.5 mL cyrovials). Perform QC of stock cultures concurrently with freezing (see section A2: QC of Stock Cultures).

g. Place and store the cryovials at -70°C or below; these are the frozen stock cultures. Store stock cultures up to 18 months. These cultures are single-use only.

A2. QC of Stock Cultures.

a. Conduct QC of the pooled culture concurrently with freezing. Streak a loopful on a plate of BAP. In addition, for *S. aureus* and *P. aeruginosa*, streak a loopful onto both selective media (MSA and Cetrimide); for *S. enterica*, streak a loopful onto XLD. Incubate all plates at 36 ± 1ºC for 24 ± 2 hours.

b. Following the incubation period, record the colony morphology as observed on the BAPs and selective media plates (including the absence of growth) and Gram stain. See Attachment 1 for details on cell and colony morphology, colony characteristics on selective media, and stain reactions.

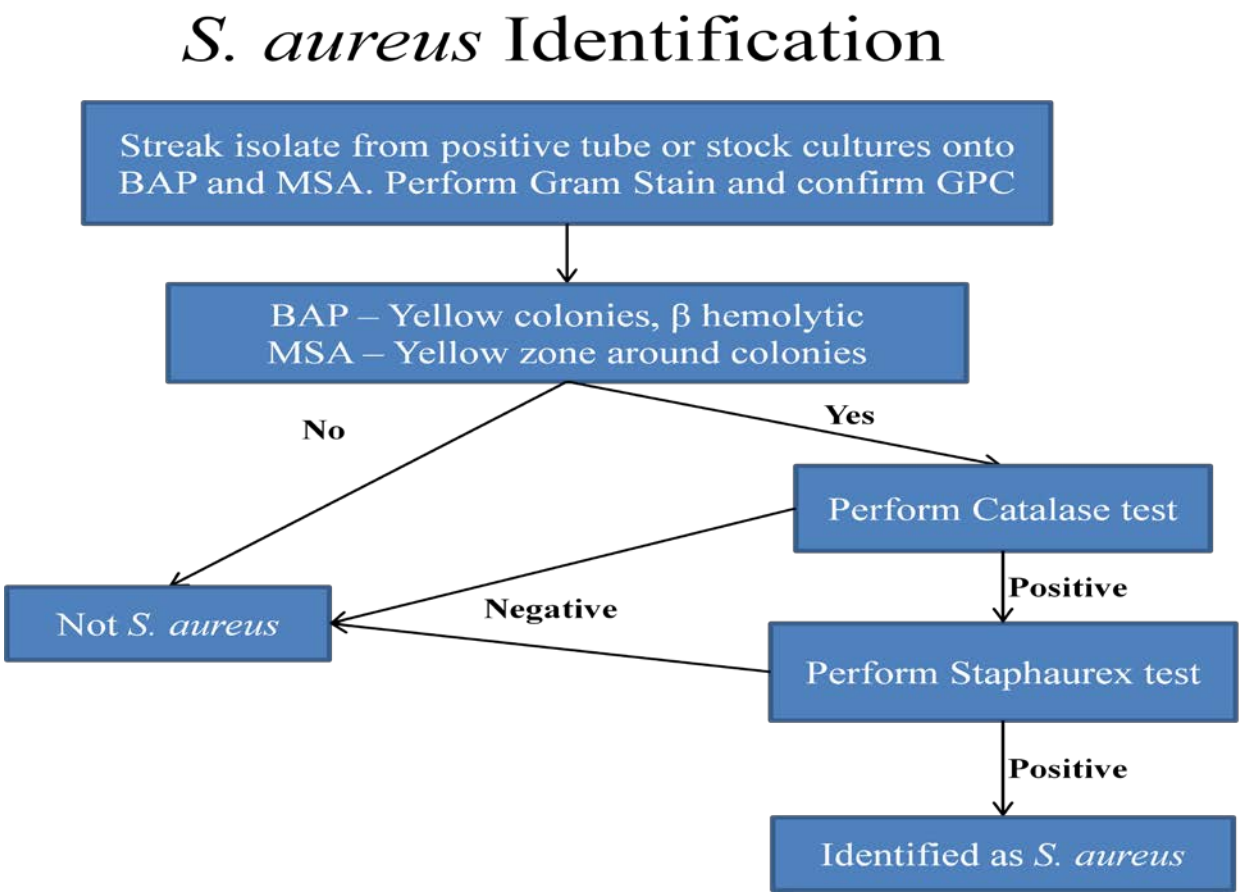
c. For each organism, perform a Gram stain (refer to 15.5) from growth taken from the BAPs according to the manufacturer's instructions. Observe the Gram reaction by using brightfield microscopy at 1000X magnification (oil immersion).

d. For additional confirmation steps refer to the appropriate Confirmation Flow Chart for *S. aureus*, *P. aeruginosa*, and *S. enterica* (see Attachment 3). Refer to 15.6-15.9 for instructions.

e. Record all confirmation results on the Test Microbe Confirmation Sheet (Quality Control) (see section 14).

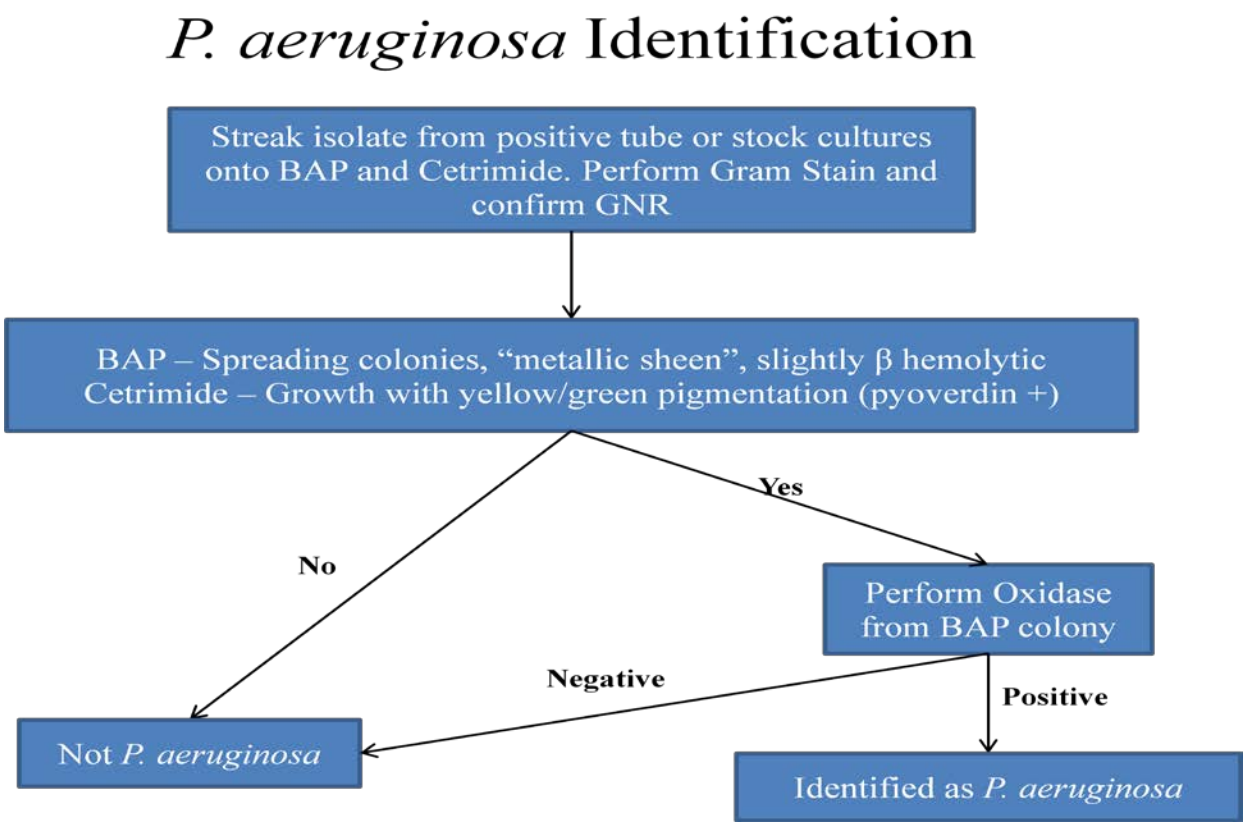
Attachment 3

Confirmation Flow Chart for *S. aureus*



Attachment 3 (cont.)

Confirmation Flow Chart for *P. aeruginosa*



Attachment 3 (cont.)

Confirmation Flow Chart for *S. enterica*